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"On the Proteid Reaction of Adamkiewicz, with Contributions to the Chemistry of Glyoxylic Acid."

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In 1874 Adamkiewicz* described the now familiar reaction which results in the production of a violet colour when strong sulphuric acid is added to the solution of a proteid in glacial acetic acid. Adamkiewicz did not apparently look upon the employment of the acetic acid as introducing anything beyond a certain modification of the action of sulphuric acid. His original communication opens with a description of the colour phenomena seen when egg-white is dissolved in strong sulphuric acid: and he begins the description of this reaction, since associated with his name, by speaking of "a special influence which the presence of glacial acetic acid has upon the colour of the sulphuric acid proteid solution." The view has since been generally held that the coloured product of the reaction arises entirely from the proteid molecule itself, as the result of an interaction between precursors liberated under the influence of the strong acids employed.

V. Udranszky† believed that the colour change which occurs is, as a matter of fact, to be classed as a furfurol reaction. It is therefore to be compared with the result of such a procedure as that of Molisch's test, in which β-naphthol and sulphuric acid are added to a proteid solution. While in the latter the added naphthol is held to react with furfurol from the proteid; in the Adamkiewicz reaction both the furfurol and a substance capable of reacting with it are supposed to be liberated from the proteid molecule. Such we believe is the prevalent view. Of late years, the Adamkiewicz reaction has been much employed as giving evidence for the presence of carbohydrate groups in certain proteid derivatives, and of its absence from others. More than one writer,‡ however, has referred to an element of uncertainty in the reaction, and it is easy to gather from the literature that this has been commonly observed.§

In what follows it will be shown that the mechanism of the reaction has been wholly misunderstood. Proof will be given that the use of acetic acid introduces an extraneous and perfectly specific factor into the reaction, involving the addition of a substance quite necessary to the formation of the coloured product. This substance, moreover, is not acetic acid itself but an impurity, which, though very generally present, is admixed in varying quantity, and is occasionally absent.

I. *The Reaction due to an Impurity in Acetic Acid.*

We were led to pursue the following investigation by observing that, with a specimen of acetic acid in use in this laboratory last year, it was impossible under any circumstances to obtain the Adamkiewicz reaction.

No matter what form of proteid might be employed, when its solution in this acetic acid was mixed with sulphuric acid, a yellow or brown, slightly fluorescent mixture was all that could be obtained. No modification in the order of the procedure, or in the proportion of the two acids employed, resulted in the production of any trace of red or violet colour.

We afterwards obtained a number of specimens of acetic acid from various makers, and were surprised to find that no small proportion of these gave equally negative results; while, of the remainder, some yielded a much more intense reaction than others, although employed under precisely similar conditions.

Either, therefore, the negative result with particular specimens was due to the presence of some impurity capable of interfering with the production of colour, or the reaction itself must be due to a substance commonly, though not universally, present as an impurity in acetic acid.

We soon obtained evidence that the latter alternative must be accepted. For we found that whenever a specimen of glacial acetic acid yielding a positive result is partially crystallised by freezing, the power to yield the reaction is diminished in the crystals and increased in the mother liquor. It is possible indeed, by repeated recrystallisation, to obtain glacial acid wholly incapable of giving the reaction.

Much more readily, however, is the reactive substance to be concentrated by distillation. Any specimen of glacial acetic acid, if distilled, will yield the whole of any chromogenic substance it may contain in the first runnings. After concentration to about half-bulk—more or less according to the proportion of reactive substance originally present—the residue will yield no trace of red or violet colour when mixed with proteid and sulphuric acid; while, on the other hand, the distillate twice or thrice fractionated yields the reaction with greatly increased intensity.*

It is easy to understand, therefore, why different specimens of acetic acid obtained in the market yield the reaction with different degrees of intensity, as this will depend upon the stage at which they were collected during distillation in bulk. It is also clear why the reaction has been looked upon by different observers as an uncertain one.

The accepted view, that the colour phenomenon is due to the interaction of two chromogenic groups, both derived from the proteid molecule under the action of the mixed sulphuric and acetic acids, is certainly erroneous. One factor necessary to the reaction is supplied by a substance admixed with the acetic acid. That it is in no sense a furfurol reaction is indicated by the fact that the addition of furfurol confers no power of yielding the colour with proteid upon a specimen of acetic acid previously without it; and, on the other hand, when furfurol is added to acetic acid containing the chromogenic substance in abundance there is equally a complete absence of the reaction upon mixing with strong sulphuric acid.

II. *Nature of the Substance responsible for the Reaction.*

Our earlier attempts actually to isolate the active substance from acetic acid by fractional distillation were unsuccessful; and, having regard to the fact that, in a reagent so familiar as acetic acid, no admixture could well have been hitherto overlooked unless the substances were present in very small amount, we determined to seek first for indirect evidence, such as might give at least some indication as to the kind of substance we had to deal with.

To this end we set out to add to acetic acid, previously deprived by distillation of its chromogenic admixture, various compounds of typical constitution, in the hope that we might find among these some that would yield at least an analogous reaction.

Wholly negative results were obtained with various homologous fatty acids; with formic, acetic, and propionic aldehydes; with acetone, and with various ethereal acetates and other esters.

But, during this preliminary stage of our investigation, the interesting observation was made that formic acid, prepared from pure glycerin and pure oxalic acid, and used instead of acetic acid under the ordinary conditions necessary for the reaction, may yield the colour in a perfectly typical manner; the spectroscopic absorption of the product obtained being identical with that seen when acetic acid is used. But from the formic no less than from acetic acid, the chromogenic substance may be distilled off, appearing always in the earlier portions of the distillates, and leaving the remainder of the formic acid to yield wholly negative results.

This result—the explanation of which becomes clear in the sequel—appeared to limit somewhat the ground we had to traverse in our search.

A further and still more definite limitation came to light when we found that the reactive substance in acetic acid is not an impurity of wholly extraneous origin, but is a derivative of acetic acid itself. When a quantity of acetic acid wholly free from the reactive substance has stood for a few weeks, a reaction may always be obtained once more from the earliest portions of a distillate; and, after standing for a month or two, even the bulk may yield a colour of moderate intensity. (*Cf. infra.*)

When, again, a pure acetate, and especially calcium acetate, is distilled with excess of sulphuric acid, the first runnings always give a marked Adamkiewicz reaction, though later portions give none. This is true even when the acetate has been made by neutralising acid which was itself wholly incapable of giving a reaction.

Lastly, among the products of the dry distillation of most acetates small quantities of a substance are found which react with proteid in a typical manner. In the case of calcium acetate the reaction obtainable is a marked one—though, as stated above, the active substance is certainly not acetone—while with an aqueous extract of the products of the dry decomposition of mercuric (not mercurous) acetate the reaction with proteid is intense.

With such indications as these facts afforded, we now fortunately elected to experiment with various two-carbon compounds of typical structure, such as might conceivably arise from acetic acid, by oxidation or otherwise.

The first positive evidence came to light when we set out to prepare glycollic aldehyde by Fenton's method.† As a mere preliminary observation, we oxidised tartaric acid in solution, by means of peroxide of hydrogen in the presence of a little ferrous sulphate, without taking especial care to keep the mixture at 0°, and without attempting to separate the dioxymaleic acid formed. A little of the oxidised solution

* 'Pflüger's Archiv,' 1874, vol. 9, p. 156.

† 'Zeitsch. f. physiol. Chem.,' 1888, vol 12, p. 395.

‡ *Cf.* Halliburton, 'Schäfer's Text Book of Physiology,' vol. 1, p. 47.

§ *Cf.* Sa'kowski, 'Zeitsch. f. physiol. Chem.,' vol. 12, pp. 220, 222.

* This applies to glacial acid; with dilute acid of lower boiling point, concentration of the product by distillation is less easy.

† 'Journ. Chem. Soc.,' 1895, vol. 67, p. 778.

was heated direct on the water bath till all evolution of CO_2 had ceased, and then cooled. A trace of Witte's peptone was added to the solution, which was free from excess of peroxide, and then strong sulphuric acid. An intense colour reaction was obtained exactly similar to that seen in a normal Adamkiewicz reaction when carried out with acetic acid rich in the chromogenic substance. The solution gave also in the spectroscope an exactly similar absorption band.

We found subsequently, however, that glycollic aldehyde, isolated, either in the syrupy or crystalline condition,* and whether in aqueous or acetic acid solution, gave no colour reaction under like conditions, but yielded only a charred product when the sulphuric acid was added. Moreover, acetic acid, however rich in the chromogenic substance, never reduces (after neutralising) alkaline copper solutions. A reduction of ammoniacal silver solutions may be obtained, but never any effect upon Fehling's solution.

We came to the conclusion, therefore, that the substance sought must be an oxidation product of glycollic aldehyde; and we now found that the latter needs only to be treated by Fenton's oxidation method carried out at the temperature of the water bath to give a product, which, when free from excess of peroxide, yields in acetic or aqueous solution the proteid reaction abundantly.

At this time we made another observation of the greatest assistance to our inquiry, finding that the chromogenic substance is produced in abundance when oxalic acid is reduced in aqueous solution by means of zinc and sulphuric acid, or, more conveniently, by sodium amalgam. The reduction need last for a few minutes only, and a little of the solution, without further treatment, will then be found to give an intense colour with proteid and sulphuric acid, the product showing spectroscopic appearances identical with those of the ordinary Adamkiewicz reaction.

There was now no doubt that a colour reaction, not to be distinguished from that of Adamkiewicz, is yielded by a substance which is at once an oxidation product of glycollic aldehyde and a reduction product of oxalic acid. It was difficult to see how this substance could be other than glycollic acid, glyoxylic acid, or glyoxal.

Pure glycollic acid was obtained from Merck. It gave no trace of a colour reaction with proteid solution and sulphuric acid. The product of its oxidation by Fenton's method reacted, however, in a perfectly typical manner, and Fenton and Jones have found that this product is glyoxylic acid.

The latter was therefore prepared from alcohol by the method of Debus. The calcium glyoxylate first obtained was recrystallised thrice. A minute crystal of the salt dissolved in water, together with a little proteid, gave, upon the addition of strong sulphuric acid, a vivid colour reaction not to be distinguished, spectroscopically or otherwise, from the reaction of Adamkiewicz.

Glyoxal, prepared subsequently from the products of the same Debus oxidation, gave no trace of such a reaction.† When glyoxylic acid is added to glacial acetic acid, previously deprived of its chromogenic power by distillation, further distillation now yields a distillate which reacts typically, and the glyoxylic acid comes over characteristically, like the original chromogenic substance in the earlier fractions.

III. *Glyoxylic Acid from Acetic Acid.*

It now became necessary to ascertain whether glyoxylic acid is, as a matter of fact, present in such specimens of acetic acid as yield the Adamkiewicz reaction.

In seeking for evidence as to this, it was necessary to remember that exceedingly little glyoxylic acid is necessary to the reaction. With an aqueous solution of such strength as will give no more than an opalescence with phenyl hydrazine, the colour reaction with proteid is well marked.

It was found, however, that oxidation with hydrogen peroxide confers abundant chromogenic power upon acetic acid previously giving no proteid reaction; and it was our first endeavour to ascertain whether, as a result of this, glyoxylic acid is produced in quantity sufficient for its easier identification.

The presence of small quantities of ferrous iron accelerates the oxidation, and is, perhaps, essential to it.‡ The process occurs most rapidly at boiling temperature, and proceeds most satisfactorily when the acetic acid is repeatedly distilled with the peroxide. The limit of the oxidation is in any case soon reached. Using twenty volumes strength, the peroxide is found to be rapidly destroyed till a volume

* Fenton and Jackson, 'Journ. Chem. Soc.,' vol. 75, p. 575, 1899. We were indebted to Mr. Hy. Jackson for a supply of the crystalline aldehyde.

† Many specimens of commercial glyoxal give the reaction, but only, as we have found, when they contain glyoxylic acid; preparations of glycollic acid may contain traces of the latter.

‡ We have found that some specimens of peroxide bring about the oxidation without the addition of iron; others undoubtedly act much less readily, unless a ferrous salt is added. While we have been unable to detect the presence of iron in the former, so small a quantity appears to affect the reaction that it is possible a trace of the metal present as an impurity may account for the difference.

has been added about equal to that of the acetic acid taken; after this the reaction becomes very slow.

We proceeded as follows:—A litre of glacial acetic acid was mixed with an equal bulk of twenty-volume peroxide and some ammonio ferrous sulphate added (half a gramme per litre, or less). The mixture was slowly distilled nearly to dryness, and the distillate returned and again distilled. The second or third distillate usually showed freedom from peroxide when tested with chromic acid; if not, distillation was repeated.

One-tenth of the final distillate was set aside, and the remainder neutralised with potash. The still acid portion being then mixed with the rest, the whole was distilled as low as possible, avoiding, however, any separation of potassium acetate in the retort. The distillate always gave an abundant proteid reaction, and if any trace of free peroxide had been left at the previous stage, it always disappeared during the distillation of the partially-neutralised mixture as just described. A small trace of free peroxide will interfere with the proteid reaction. On adding phenyl hydrazine hydrochloride (without acetate) to the distillate thus obtained, a light yellow precipitate begins to separate almost at once, and after standing it becomes considerable in amount, and is crystalline. But although, as we were able to show, the hydrazone of glyoxylic acid is present in this precipitate, it is mixed with a considerable proportion of a compound much less soluble in acetic ether and in hot water. If the original precipitate be recrystallised from a minimal quantity of acetic ether, the substance which separates first consists of perfectly colourless glistening plates, which after recrystallising from acetic ether may assume the form of rosetted prismatic needles. These melt sharply at 184°.

The nature of this substance became clear after the publication of certain recent observations. Gerhard Ollendorff has shown that formic aldehyde is formed when glycollic acid is oxidised with peroxide of hydrogen, and Fenton* calls attention to the fact that glyoxylic acid must in this case be the intermediate product. The product we obtained from acetic acid was undoubtedly the compound of formaldehyde described by Wellington and Tollens.†

A portion repeatedly recrystallised from acetic ether and showing a constant melting point (184°) was analysed.

0·147 gramme gave 27·4 c.c. moist N, at 12°, and 758 mm. N = 22·07 per cent.

Another preparation, recrystallised from a mixture of alcohol and toluol, melted at 182—183°; of this

0·211 gramme gave 39·3 c.c. moist N, at 14°, and 758 mm. N = 21·87 per cent.

I.	II.	Calculated for $C_{15}H_{16}N_4$.
22·07	21·87	22·22

This hydrazone can be obtained pure in the above manner with great ease if not more than 4 to 5 grammes of phenylhydrazine hydrochloride have been added to the final distillate obtained after oxidising, as above, 1 litre of acetic acid, nearly neutralising the mixture and distilling. We prepared the compound from formaldehyde, and found it to agree with our product in every particular.

Formaldehyde certainly does not yield the proteid reaction, and its formation when acetic acid is treated as described seems to be in itself evidence for the formation of glyoxylic acid during the process, as it is difficult to see how it could arise during the oxidation of acetic acid if not from a preliminary formation of glyoxylic acid with subsequent loss of carbon dioxide.

But its formation adds greatly to the difficulty of obtaining pure the hydrazone of glyoxylic acid itself, especially as the precipitate produced by phenylhydrazine undoubtedly contains, in addition to the compound of Wellington and Tollens, smaller amounts of the derivatives described by J. W. Walker.‡

After the nature of this bye-product was recognised we modified our procedure by neglecting the earlier portions of the final distillate which contains, of course, the greater part of the formaldehyde. Phenylhydrazine hydrochloride added to the latter half, or two-thirds, of such a distillate yields a precipitate which forms more slowly than that obtained when the whole is dealt with. After twenty-four hours it is usually crystalline and of a yellow colour, growing darker with further standing.

We found it easier to obtain a product with a constant melting point by recrystallising from hot water rather than from an organic solvent, prolonged heating with the water being at any stage avoided. This treatment involves considerable loss, however, and we obtained only about 4 decigrammes of the hydrazone after oxidising 3 litres of acetic acid. This, however, had all the characters of glyoxylic hydrazone, and melted sharply at 137°.

0·204 gramme gave 30·4 c.c. moist N at 16° and 750 mm. N = 17·14 per cent. Calculated for $C_8H_8O_2N_2$ = 17·07.

* Fenton, 'Journ. Chem. Soc.,' 1900, vol. 77, p. 1296.

† 'Deutsch. Chem. Ges. Berichte,' 1885, vol. 18, p. 3330

‡ 'Journ. Chem. Soc.,' 1896, vol. 69, p. 1280.

When acetic acid has been oxidised as described and the mixture partially neutralised and distilled, the distillate, when treated with excess of chalk, will yield, after standing and filtering, the reaction for glyoxylic acid described by Perkin and Duppa. If after treatment with chalk a slight excess of calcium hydrate be added, and the mixture concentrated *in vacuo* to about one-third its original bulk, this reaction with aniline oxalate is obtained in a highly characteristic manner.

The methods we have hitherto employed do not yield the glyoxylic acid in solutions of sufficient strength to permit of its calcium salt being separated from the associated acetate and isolated in substance. The ease with which the salt dissociates and the volatility of the acid with water vapour make concentration of small avail.

The evidence for the formation of glyoxylic acid during oxidation appears, however, to be conclusive, and it is interesting to note that, judging from the gradual development of the reaction with proteid, this oxidation goes on slowly when acetic acid is exposed to air, and especially under the influence of light. Ferrous iron undoubtedly accelerates this, and if acetic acid giving no proteid reaction be somewhat diluted, and a little ferrous salt added, exposure to direct sunlight will confer a reactive power in the course of a few hours.

We have not been able to separate the hydrazone in quantity sufficient for its identification from average specimens of untreated acetic acid; but it appears equally difficult to do so when small quantities of glyoxylic acid, sufficient to confer an average chromogenic power, have been added to a specimen previously giving no reaction.

On one occasion we obtained a quantity of glacial acid giving the reaction with special intensity. This acid had crystallised in bulk, and we were supplied with drainings from the crystals. Seven litres were fractionally distilled until the chromogenic substance was concentrated into about 1 litre. This was nearly neutralised and again distilled. Phenylhydrazine acetate added to the distillate gave a considerable quantity of crystalline precipitate, yellow at first, darkening on standing. This was obtained before we had identified glyoxylic acid as the substance sought, and most of the hydrazone was lost in preliminary solubility tests. A small quantity was reserved, however, and this, recrystallised thrice from hot water, melted sharply at 137°.

The observations we have hitherto made give no quantitative indications of any value. In this paper we have been mainly concerned with the endeavour to prove the nature of the active substance in the proteid reaction. We propose to study the oxidation of acetic acid further, and to define if possible the conditions necessary for a maximal yield of glyoxylic acid.

IV. *Remarks on the Colour Reaction: Spectroscopic Phenomena.*

Adamkiewicz* observed that the colour produced in the reaction varies from red to violet, the blue element increasing with increase in the amount of acetic acid employed. When glyoxylic acid in aqueous solution is used, unless the solution be very dilute, the colour partakes more of a blue shade than is usually seen with ordinary specimens of acetic acid. But after concentrating the reactive substance of the latter by fractional distillation (*supra*) or upon large dilution of the glyoxylic acid solution, the colours obtained become identical. The spectroscopic absorption is identical whichever reagent is employed.

When sulphuric acid is added to a solution of proteid in acetic acid wholly free from glyoxylic acid, a considerable amount of charring occurs, and the mixture becomes somewhat fluorescent. When, under similar circumstances, very little glyoxylic acid is present, the reddish colour obtained is still associated with fluorescence. But, when sufficient of the glyoxylic acid is present, whether in acetic or aqueous solution, to combine with the whole of the proteid product concerned in the reaction, there is complete absence of charring and little or no fluorescence. The solution becomes of a pure violet-blue colour.

The coloured product of the Adamkiewicz reaction is usually stated to show an absorption band between *b* and F in the position of the urobilin band; and Krukenberg described another between D and E. Salkowski found the former to be inconstant, and we are convinced that the latter alone is proper to the real product of the colour reaction: the former, when seen, being due to some accessory effect of the strong acids upon proteids. It is never seen in the original form of the reaction unless the acetic acid employed is greatly deficient in reactive power, and it is not observed with glyoxylic acid. The other band is always present, and is identical after the use of a satisfactory specimen of acetic acid and when a solution of glyoxylic acid is used.

The band shrinks rapidly from its more refrangible edge on dilution of the solution, its redward edge shifting but little.

The following readings show the correspondence seen after employing acetic acid as obtained in the market (but with its active substance concentrated by distillation) and that seen after the use of glyoxylic acid in aqueous solution. The strengths were so arranged that, before dilution, the colour of each solution appeared to be of the same

* *Loc. cit.*, p. 158.

intensity. Witte's peptone was the proteid employed to obtain the reaction:—

	Acetic acid.	Aqueous glyoxylic acid.
Strong	λ 480—λ 625	λ 480—λ 630
Diluted with an equal volume of sulphuric acid	λ 495—λ 625	λ 495—λ 625
Diluted with thrice its volume of sulphuric acid	λ 530—λ 610	λ 530—λ 615

V. *Other Sources of the Reactive Substance.*

Of the typical two-carbon compounds—glycol, glycollic aldehyde, glycollic acid, glyoxal, glyoxylic acid, and oxalic acid—none but the aldehyde-acid (glyoxylic acid, HCO.COOH or $CH(OH)_2.COOH$), gives the smallest indications of yielding a colour-reaction with proteid on addition of sulphuric acid. It would seem that the reaction is not common to aldehyde-acids, as glycuronic acid, $HCO(CH.OH)_4COOH$, gives wholly negative results. Again, a ketonic acid so closely related to glyoxylic acid as pyruvic acid, $CH_3.CO.COOH$, gives no indication of a reaction.

Glyoxylic acid stands, of course, alone in containing the aldehydic and carboxylic groups in juxtaposition. Our observations are far from being complete enough to enable us to assert that a reaction with proteid of the special type under consideration depends essentially upon this particular structure. But the preliminary observations we have made tend to give some probability to this view. At least it may be said that hitherto we have never obtained a reaction except with products in which either glyoxylic acid has been shown to be present, or in which its presence is extremely probable.

For instance, we have found that mesoxalic acid (prepared from barium alloxanate) in aqueous solution gives with proteid and sulphuric acid a perfectly typical Adamkiewicz reaction; but under the conditions employed we have found that a portion at least of the mesoxalic acid present loses carbon dioxide, so that it is in the highest degree probable that glyoxylic acid is in this case also the substance which reacts.

Pyruvic acid gives, as we have said, no trace of a reaction, but the product of its oxidation by peroxide of hydrogen undoubtedly reacts. *Paralactic acid*, itself inactive, yields also an active product on oxidation by Fenton's method at the temperature of the water bath. These two cases go together, as Fenton and Jones have shown that lactic acid yields pyruvic acid when oxidised at 0° in the presence of ferrous iron. It seems extremely probable that the oxidation of the pyruvic acid at the higher temperature yields mesoxalic acid, and that the reaction obtained is therefore due in each case to glyoxylic acid.

One abundant source of a reactive substance is found in the oxidation of *glycerin* by Fenton's method, carried out at the temperature of the water bath. *Glyceric acid* yields the substance under like conditions; and, as Fenton and Jones* have shown that glyceric acid, when the oxidation is carried out in the cold, gives a product which is almost certainly either hydroxy-pyruvic acid or the tautomeric substance dihydroxyacrylic acid, the facts here quite probably fall into line with those just enumerated. The substance which reacts with proteid is only obtained in quantity in these cases when the oxidation is carried out at higher temperatures than those used by Fenton, and if the oxidised products are distilled, it is always found that the distillate gives the reaction.

When *dextrose* solutions are boiled with peroxide in the presence of ferrous salts a substance is formed, volatile with steam, which yields the proteid reaction abundantly. Preliminary observations that we have made leave little doubt that this is glyoxylic acid itself.

If it should prove that the reaction is, as a matter of fact, peculiar to glyoxylic acid, it certainly forms a very delicate test for that substance.

VI. *Glyoxylic Acid Solutions a Practical Test for Proteids.*

By replacing the acetic acid of the Adamkiewicz reaction by weak aqueous solutions of glyoxylic acid a very beautiful and reliable test for proteids is obtained. The colour reaction is brilliant, and the test is, of course, subject to none of the uncertainty inseparable from the use of acetic acid.†

* 'Journ. Chem. Soc.,' 1900, vol. 77, p. 72.

† It is certainly rare to find a specimen of acetic acid which yields no reaction, though many contain too little glyoxylic acid to give a satisfactory colour. It seems to be possible, however, that there have been cases of a proteid derivative being found to yield no Adamkiewicz reaction, in which the negative result was really due to the acetic acid employed. We have, for instance, prepared and carefully purified the primary albumoses from Witte's peptone by the method of E. P. Pick ('Zeitsch. f. physiol. Chem.,' 1899, vol. 28, p. 219). Unlike this observer, we have found these products to yield a marked Adamkiewicz reaction; and with all reserve, we venture to suggest that the acetic acid employed by Pick at this stage of his observations may have chanced to be free from chromogenic power.

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In preparing such a test solution, there is usually no need to separate the glyoxylic acid from associated products. Excellent test solutions may be made by oxidising upon the water bath, in the presence of small quantities of ferrous iron, either weak solutions of tartaric acid or mixtures of glycerin and water, great care being taken to ensure that no trace of free peroxide remains at the close of the operation. But we strongly recommend the use of reduced oxalic acid for the purpose, as a solution can be prepared with great ease, and almost without regard to conditions. In a moderately strong solution of oxalic acid a few lumps of sodium amalgam are placed, the amount taken of the latter being less than sufficient to neutralise the acid. When the evolution of hydrogen has ceased, the solution is poured off from the mercury and filtered. It will be found, even after large dilution, to yield an intense reaction with proteids if used instead of acetic acid under the familiar conditions of the Adamkiewicz test. The proteid, or the proteid solution to be tested, should be first added to the reagent, and then strong sulphuric acid poured down the side of the test tube. The reaction may be first observed at the junction of the fluids and the latter subsequently mixed. At least one-third volume of sulphuric acid should he used, but the quantity may be almost indefinitely increased. There is no tendency to charring.

Summary.

The proteid reaction described by Adamkiewicz is not a furfurol reaction, but depends upon the presence of small quantities of an impurity in the acetic acid employed. Some specimens of acetic acid yield no reaction, and all may be deprived of chromogenic power by distillation.

The substance essential to the reaction is glyoxylic acid.

Small quantities of glyoxylic acid are produced during the oxidation of acetic acid by hydrogen-peroxide in the presence of ferrous iron. Under the conditions used in this research, part of the glyoxylic acid thus formed is split up, yielding formaldehyde.

Glyoxylic acid is slowly formed when acetic acid stands in the air, and more rapidly in the presence of ferrous iron and under the influence of direct sunlight. Most specimens of acetic acid contain small amounts of glyoxylic acid as an admixture.

A dilute aqueous solution of glyoxylic acid, which may be readily prepared by the reduction of oxalic acid with sodium amalgam, forms an admirable test for proteids when used instead of acetic acid under the ordinary conditions of the Adamkiewicz test.

In carrying out this investigation we have been led to employ extensively the method of oxidation described by H. J. H. Fenton, and as a result we have in some degree trenched upon the systematic study of the oxidation of organic acids which he has in hand. It is with his consent that such of our observations are published.

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